REMARKS

These remarks are in response to the Office Action mailed June 2, 2003. The specification has been amended to delete the browser-executable hyperlink found in paragraphs 148, 172, and 220. Claims 32, 34-38, 42-44, and 46 have been amended to more particularly define Applicants' invention and to correct the dependencies of certain dependent claims. In addition, support for this amendment to claim 38 is found in the specification at paragraph 130, page 52, and at paragraph 132, page 53. Thus, the amendments introduce no new matter. Claims 17, 32-40 and 42-46 are pending.

Responsive to the Examiner's objection to the drawings, corrected formal drawings are filed herewith as Exhibit A. Specifically, Figure 1 has been amended to remove the black shading therein. Accordingly, reconsideration and withdrawal of the objection to the drawings are respectfully requested.

A. Rejection Under 35 U.S.C. § 112, first paragraph

The rejection of claims 17 and 32-39 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was allegedly not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention, is respectfully traversed.

The burden of demonstrating that the claims are allegedly not supported by an adequate written description falls on the Examiner. *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976). In this case, the Examiner has provided insufficient evidence to call into question the written description set forth in the present application. Accordingly, for the following reasons, it is respectfully submitted that the Examiner has not met the burden of demonstrating an alleged lack of written description for the claimed invention.

Applicants respectfully disagree with the Examiner's assertion that the rejected claims contain "no identifying characteristics . . . with respect to the type of 'active target members' (e.g., the specific type of protein or the protein sequence) or . . . a common functionality for the conjugation of the probe and the active target members (e.g., the sequence for the binding site of the 'active target members' to the probe) and the probes specific to the active target members," citing *University of California v. Eli Lilly and Co.*, 43 USPQ2d 1398, 1405 (1997). In response to this assertion, Applicants respectfully direct the Examiner's attention to *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 :

> "The written description requirement may be satisfied through sufficient description of a representative number of species..." (emphasis added).

Moreover, it is submitted that an adequate description of a "representative number of species" does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces. *In re Bell,* 991 F.2d 781, 785 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) With respect to the active target members set forth in the specification, it is submitted that the specification clearly describes a "representative number of species" to demonstrate that Applicants were in possession of the claimed active target members.

The present invention is drawn to methods for determining in a plurality of proteomic mixtures the presence of active target members of a group of related proteins in each of the proteomic mixtures. As such, the present invention provides methods for determining changes in protein, activity rather than simply variations in levels of protein expression (i.e., amount of protein present).

It is well-established that claims are to be interpreted in light of the specification (*AFG Industries, Inc. v. Cardinal IG Co., Inc.,* 239 F.3d 1239, 57 USPQ 2d 1776 (Fed. Cir. 2001) The specification contains more than sufficient guidance with respect to the identifying

characteristics of "active target members." For example, at page 24, paragraph 74, the specification describes examples of target proteins, as follows:

> Exemplary protein targets described herein include enzymes, included in the groups oxidoreductases, hydrolases, ligases, isomerases, transferases, and lyases and include such enzymes or enzyme groups as serine hydrolases, metallo-hydrolases, dehydrogenases, e.g. alcohol and aldehyde dehydrogenases, and nucleotide triphosphate (NT)-dependent enzymes, although, the invention envisions ABPs which recognize any protein, e.g., enzyme, family. Other proteins include proteins that bind to each other or to nucleic acids, such as transcription factors, kringle structure containing proteins, nucleic acid binding proteins, G-protein binding receptors, cAMP binding proteins, etc.

Similarly, at page 24, paragraph 75, the specification provides, "Targets of interest will be particularly enzymes, other proteins include receptors, transcription factors, G-proteins, and the like." See also, page 47, paragraph 119, where the specification describes various types of enzymes that may serve as target proteins:

> Enzymes typically fall within six main classes including oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. In a particular embodiment illustrated herein, an enzyme group of interest includes the class of hydrolases. One genus of the class is serine hydrolases, which includes sub-genera such as proteases, e.g.trypsins, chymotrypsins, esterases, such as acetylcholinesterases, thioesterases, amidases, such as FAAH, and acylpeptide hydrolases, lipases, transacylases, such as lecithin:cholesterol acyltransferase. Another sub-genus is cysteine hydrolases, such as caspases, cathepsins, and palmitoyl acyltransferases. Another sub-genus is metallohydrolases, including matrix metalloproteinases ("MMPs"), e.g. MMP1 – 13, membrane type metalloproteinases, aminopeptidases, and ADAMalysins. In addition, are phosphatases, such as alkaline phosphatases, acid phosphatases, protein tyrosine phosphatases, and serine/threonine phosphatases. Further included are the GTPases and ATPases. Besides hydrolases are kinases, which include enzymes such as tyrosine

kinases, e.g. src, abl, and lck, serine/threonine kinases, e.g. MAP kinases, MAPK kinases, CAM kinases, protein kinase C, and casein kinases. Also of interest are oxidoreductases, such as cytochrome P450s, amine oxidases, alcohol dehydrogenases, aldehyde dehydrogenases, such as ALDH1, ALDH2, ALDH3, desaturases, etc. Other proteins that are of interest include receptors, such as HLA antigens, hormone receptors, G-proteins coupled receptors, ion channels, transcription factors, protein inhibitors and the like.

Additionally, the specification describes what is meant by "related group of proteins," that is, proteins that perform the same activity, and provides examples. See, for example, page 39, paragraph 104:

> The probe may be specific for a single protein or more usually a related group of proteins. By related group of proteins is intended proteins that perform the same activity, as with enzymes that belong to the same group and catalyze the same reaction, e.g. hydrolysis, phosphorylation, oxidation, etc., and usually having one or more of the following characteristics: the same functionality at the active site; the same spatial orientation of functional groups that bind to the ligand; similar spatial structure and conformation; similar molecular weight; the same or similar cofactors or complexing proteins; and similar function. To enhance the distinction between active proteins and inactive proteins, special chemically reactive groups are employed.

Thus, claims 17 and 32-29, when interpreted in light of the specification, adequately describe what is meant my "active target members of a group of related proteins."

The specification also adequately describes what is meant by "a common functionality for the conjugation of the probe and the active target members." Because the ABPs employed in the claimed methods label proteins in an activity-dependent manner, those skilled in the art recognize that each ABP will vary depending upon the target protein(s) chosen for activity-based analysis. It is respectfully submitted that the ability to label proteins in an activity-dependent manner is a readily identifiable characteristic that, combined with the abundant

information provided with respect to selection of target proteins and characterization of functional groups of activity-based probes directed to various target proteins, is sufficient to demonstrate possession of the claimed invention. The specification provides, for example, at page 27, paragraph 81, that "a functional group (F) . . . specifically and covalently bonds to the active site of a protein." The specification also provides examples of particular functional groups, for example, at page 28, paragraph 83:

> Exemplary Fs as used in an ABP of the invention include an alkylating agent, acylating agent, ketone, aldehyde, sulphonate or a phosphorylating agent. Examples of particular Fs include, but are not limited to fluorophosphonyl, fluorophosphoryl, fluorosulfonyl, alpha-haloketones or aldehydes or their ketals or acetals, respectively, alpha-haloacyls, nitriles, sulfonated alkyl or aryl thiols, iodoacetylamide group, maleimides, sulfonyl halides and esters, isocyanates, isothiocyanantes, tetrafluorophenyl esters, N-hydroxysuccinimidyl esters, acid halides, acid anhydrides, unsaturated carbonyls, alkynes, hydroxamates, alpha-halomethylhydroxamates, aziridines, epoxides, or arsenates and their oxides. Sulfonyl groups may include sulfonates, sulfates, sulfinates, sulfamates, etc., in effect, any reactive functionality having a sulfur group bonded to two oxygen atoms. Epoxides may include aliphatic, aralkyl, cycloaliphatic and spiro epoxides, the latter exemplified by fumagillin, which is specific for metalloproteases.

Additionally, the specification provides guidance on the selection of an appropriate functional group for protein target, for example, at page 39, paragraph 105:

> A "chemically reactive group" is a moiety including a reactive functionality that does not react efficiently with the generally available functional groups of proteins, e.g. amino, hydroxy, carboxy, and thiol, but will react with a functionality present in a particular conformation on a surface. In some situations the reactive functionality will serve to distinguish between an active and an inactive protein. In other situations, the conformation of the chemically reactive group will bind to the specific conformation of the target protein(s), whereby with a slowly reactive functionality or one that requires activation, the predominant reaction will be at the active site. For example a

photoactivatable group may be used such as a diazoketone, arylazide, psoralen, arylketone, arylmethylhalide, etc. any of which can bind non-selectively to the target protein, while the probe is bound to the active site. Olefins and acetylenes to which are attached electron withdrawing groups such as a sulfone, carbonyl, or nitro group may be used to couple to sulfhydryl groups.

The specification provides much guidance with respect to the selection of "active target proteins." For example, the specification provides at page 46, paragraph 118:

> For many of the enzyme genera, functionalities are known that do not significantly react with enzymes of other genera, particularly non-enzymatic proteins and enzymes that have different reactive sites. It is also desirable that the functionality does not react with inactive target enzyme. Examples of inactive states include: 1) proenzymes, e.g. requiring cleavage of the protein; 2) enzymes bound by endogenous inhibitors (either covalent or non-covalent); 3) enzymes in an inactive conformation (e.g. enzymes that require the binding of another protein, a conformational change, covalent modification by phosphorylation/reduction/oxidation/methylation/acylation (e.g. formic or acetic acid) for conversion to an active state; 4) denatured enzymes; 5) mutant enzymes; 6) enzymes bound by either reversible or irreversible exogenous inhibitors; and 7) enzymes requiring a cofactor for activity. The enzymes of interest will usually have at least one of serine, threonine, cysteine, histidine, lysine, arginine, aspartate or glutamate as a member of the active site involved in the catalysis of the enzyme reaction. One or more of the functionalities of these amino acids may be the target of the ABP. The manner in which the inactive enzyme is inactivated is chosen to emphasize the differences in bonding of the ABP between the active and inactive state.

Thus, the specification provides adequate guidance for selection of common functionalities.

Additionally, the specification provides a description of a method for determining the enzymes and/or active sites to which activity-based probes bind (page 47, paragraph 120):

The enzymes and/or the sites to which the ABPs bind may be identified in a variety of conventional ways, such as isolating the enzyme, e.g. using an affinity matrix, and characterizing it by mass spectrometry, isolating and sequencing the enzyme or proteolytically fragmenting the enzyme and determining the fractions as a profile for a specific enzyme, electrophoretic separation and Western blotting, or immunoassays employing labeled antibodies specific for the enzyme. The conditions under which the binding is determined will generally be mild conditions, conveniently ambient, using a buffer solution, where the buffer concentration will generally be in the range of about 50 – 200mM and the concentration of each active enzyme will generally be about 0.01 pg (picograms)/ml to 0.1 mg (milligrams)/ml. After sufficient time for enzyme binding, non-specifically bound enzyme may be washed away. One may wish to use conditions of increasing stringency, by increasing salt concentration, organic solvent, temperature, etc., to determine levels of binding affinity. By comparison of the sequences at different levels of affinity, one may readily optimize the affinity sequence. In this way one or more libraries of affinity moieties are developed and can be used in conjunction with the other members of the ABP. By having a repertoire of affinity moieties, a specific affinity moiety can be selected that provides the least amount of background in a particular environment. For example, one affinity moiety may be preferred for a target enzyme in a particular background of other enzymes of the same genus.

Applicants also respectfully disagree with the Examiner's assertion that "specification description is directed to the syntheses of a specific probe (e.g., the biotinylated fluorophosphonate probe such as FP-biotin and FP-peg biotin) that have specificity toward an "active target member" (e.g., serine hydrolases). As noted above, the specification clearly sets forth a wide variety of proteins contemplated for use in the practice of the invention and sets forth (for exemplary purposes only) activity-based quantitation and determination of the serine hydrolases (see specification, Example 4). In addition, the specification describes several sulfonate ester activity-based probes (see, for example, Example 7) . Additionally, at page

108, paragraphs 243 and 244, for example, the specification describes methods of design and synthesis of active site-directed chemical probes that target metallo-hydrolases and probes that target nucleotide triphosphate (NT)-dependent enzymes Applicants respectfully point out that particular examples of ABPs set forth in the Examples are merely illustrative and not intended to be limiting.

It is respectfully submitted that Applicants are entitled to claims drawn as broadly as the prior art will allow. The activity characteristics set forth in the present claims are the most appropriate descriptions known to Applicants to describe the methods of the present invention. Thus, it is submitted that the specification "clearly allows persons of ordinary skill in the art to recognize that he or she invented what is claimed." *Union Oil Co. of California v. Atlantic Richfield Co.* 54 USPQ2d 1227 (2000)

Applicants submit that the present specification contains a complete description of the invention sufficient to enable a person skilled in the art to utilize the methods of the invention. Accordingly, it is respectfully submitted that the rejection of claims 17 and 32-39 under 35 U.S.C. § 112, first paragraph, as allegedly lacking adequate written description, is not properly applied. Reconsideration and withdrawal of the rejection are therefore respectfully requested.

B. Rejection Under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully disagree with the Examiner's rejection of claims 17, 32-40, and 42-46 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

The Examiner states that the term 'proteomic mixture' of claim 17 is vague and indefinite and confusing with regard to claim 36 '*wherein the proteomic mixture is in an intact cell*.' Applicants respectfully disagree with the Examiner's assertion. The specification, for

example, at page 43, paragraph 112, provides the following description of proteome and proteomic mixture:

> By a proteome is intended at least about 20% of total protein coming from a biological sample source, usually at least about 40%, more usually at least about 75%, and generally 90% or more, up to and including all of the protein obtainable from the source. Thus the proteome may be present in an intact cell, a lysate, a microsomal fraction, an organelle, a partially extracted lysate, biological fluid, and the like. The proteome will be a mixture of proteins, generally having at least about 20 different proteins, usually at least about 50 different proteins and in most cases 100 different proteins or more. In effect, the proteome is a complex mixture of proteins from a natural source and will usually involve having the potential of having 10, usually 20, or more proteins that are target proteins for the ABPs that are used to analyze the proteome profile. The sample will be representative of the target proteins of interest.

Thus, the specification clearly provides that the proteomic mixture may be present in an intact cell. It is respectfully submitted that this description conveys without ambiguity the meaning of "proteomic mixture" as recited in claims 17 and 36.

The Examiner states that the phrase "when active" is "vague and indefinite because it is unclear as to the cause of the biological activity of the 'target members.'" Applicants respectfully disagree that this phrase renders claim 17 indefinite. Because the claimed methods are directed to methods of determining the presence of active proteins, those skilled in the art recognize that ABPs employed in invention methods are specific for an active site *when active*, as opposed to being specific for proteins in which the active site is not active, for example, due to denaturating. Indeed, the subject phrase serves to distinguish over proteins that are present in a proteomic mixture, but not necessarily active. As set forth in the specification, for example, at page 24, paragraph 75:

> An "active protein" of the invention refers to a protein, e.g., enzyme, in its normal wild-type conformation, e.g. a catalytically active state, as opposed to an

inactive state. The active state allows the protein, to function normally. An inactive state may be as a result of denaturation, inhibitor binding, either covalently or non-covalently, mutation, secondary processing, e.g. phosphorylation or dephosphorylation, etc. Functional states of proteins or enzymes as described herein may be distinct from the level of abundance of the same proteins or enzymes. An active site is an available wild-type conformation at a site that has biological activity, such as the catalytic site of an enzyme, a cofactor-binding site, the binding site of a receptor for its ligand, and the binding site for protein complexes, for example. In many instances, one is interested in knowing the level of availability of such sites. Targets of interest will be particularly enzymes, other proteins include receptors, transcription factors, G-proteins, and the like.

The Examiner states with reference to claim 38, that the phrase "said target enzymes" lacks antecedent basis. Applicants respectfully disagree. At line 8 in claim 38, the phrase "a target enzyme" is recited, thereby providing antecedent basis for "said target enzymes."

The Examiner state that the definition of "X" in claim 38, renders claim 38 indefinite "because it is unclear as to how 'X' can be a ligand that binds to a complementary (e.g. definition of 'reciprocal') receptor in which the receptor is not defined. Presently amended claim 38 defines X as "a ligand for binding to a receptor or a chemically reactive functionality." The specification defines the term "ligand", for example, at paragraph 132, page 53:

> The ligand can be any ligand that does not interfere with the binding of the subject compounds to the serine hydrolases, relatively small, less than about 1kDal, frequently less than about 500Dal, has an appropriate receptor and is synthetically accessible. There are a number of popular ligands, such as biotin, dethiobiotin, deiminobiotin, digoxin, 2,4-dinitrophenyl, and derivatives thereof, fluorescein, etc. These ligands have strongly binding natural receptors, such as strept/avidin for biotin and dethio- or deiminobiotin, and antibodies for the

> remaining listed ligands. In some instances it will be desirable to release the serine hydrolase bonded to the inhibitor of this invention from the receptor. A useful pair is dethiobiotin or deiminobiotin, which can be replaced by biotin.

Indeed, it is submitted that the terms "ligand" and "receptor" are terms of art that a skilled artisan would readily recognize and understand. Thus, it is respectfully submitted that no ambiguity exists with respect to the definition of "X" in claim 38.

With specific reference to the definition of the formula (R*(F-L)-X), Applicants respectfully submit there is no ambiguity regarding the binding site for the active target member. As set forth in the specification (see, e.g., page 30, paragraph 88), it is a combination of R and F that defines the binding site for a particular set of proteins. For example, as set forth in paragraph 88, when X is biotin or any ligand, L is any linker of varied composition and length, F is a sulfonate and R is a pyridyl group, a distinct protein profile is observed as compared with the same ABP wherein the R group is methyl. Thus, in certain embodiments of the invention, by varying R when bonded to a sulfonyl group, different binding profiles will be obtained.

With specific reference to claim 36, Applicants respectfully submit that there are no missing essential steps of invention methods recited therein. The Examiner asserts that a step describing how the ABP's enter an intact cell is allegedly omitted from claim 36. However, it is respectfully submitted that this is not an essential step of the present invention methods. As set forth in the specification (see, e.g., page 42, paragraph 110), a sample (such as an intact cell) may be treated prior to employing a method of the invention. Specifically, when the probes do not readily pass through a cellular membrane, the cells may be treated with a reagent effective for lysing the cells. Applicants submit that this treatment step is not an essential step in the methods of the invention. Indeed, if necessary, this step is performed prior to employing a method of the invention. Thus, it is respectfully submitted that no essential steps are missing from the method defined by present claim 36.

For all of the reasons set forth above, it is respectfully submitted that the rejection of claims 17, 32-40, and 42-46 under 35 U.S.C. § 112, second paragraph is not properly applied. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

C. Rejections Under 35 U.S.C. § 102

The rejection of claim 17 under 35 U.S.C. § 102(e) as allegedly being anticipated by Chin, et. al., (U.S. Patent No. 6,197,599) is respectfully traversed. Applicants' invention, as defined by claim 17, distinguishes over Chin by requiring a method for determining in a plurality of proteomic mixtures the presence of active target members of a group of related proteins in each of the proteomic mixtures, the related proteins related in having a common functionality for conjugation at an active site, the method comprising combining each of the proteomic mixtures with at least one activity-based probe comprising a reactive functionality specific for the active site when active, under conditions for conjugation of the probe(s) to the target members; determining the presence of target members conjugated with the probe in each of the proteomic mixtures; whereby the presence of the target members conjugated to the probe(s) in the proteomic mixtures is indicative of the presence of active target members in the mixtures. Chin does not describe such a method. Specifically, Chin does not disclose or suggest a method for determining active target members (proteins) in a proteomic mixture. Instead, Chin merely describes methods for detecting interactions between a first protein and a second protein.

Indeed, those skilled in the art readily recognize that the methods set forth in the '599 patent do not employ activity-based probes, and therefore can not be used to profile classes of proteins based on activity. Thus, it is respectfully submitted that Chin does not describe each and every element of claim 17. Accordingly, reconsideration and withdrawal of the rejection of claim 17 under 35 U.S.C. § 102(e) are respectfully requested.

The rejection of claims 17, 32-36, 38-40, 42, and 46 under 35 U.S.C. § 102(a) as allegedly being anticipated by Liu, et. al. (PNAS, 1999, 96(26): 14694-14699), is respectfully

traversed. Applicants respectfully submit that Liu is not available as prior art under 35 U.S.C. 102(a) since the subject matter set forth in Liu was derived from Applicants' own work. Indeed, it is noted present inventors Cravatt and Patricelli are co-authors of the Liu publication, and, as set forth in the accompanying declaration, co-author Liu did not contribute to the mental conception of the present invention. Accordingly, reconsideration and withdrawal of the rejection of claims 17, 32-36, 38-40, 42, and 46 under 35 U.S.C. § 102(a) are respectfully requested.

D. Rejection Under 35 U.S.C. § 103(a)

The rejection of claims 17, 32-36, 38-40, 42, and 46 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Liu et al. (PNAS, 1999, 96(26): 14694-14699), in view of Blanchard et al. (U.S. Patent No. 5,151,164), is respectfully traversed. As set forth above and in the accompanying declaration, the primary reference Liu is not available as prior art under 35 U.S.C. § 102(a), and therefore can not be combined with Blanchard in applying a rejection under § 103(a). Further, even when taken alone Blanchard et al. does not render the claims obvious. Accordingly, reconsideration and withdrawal of the rejection of claims 17, 32-36, 38-40, 42, and 46 under 35 U.S.C. § 103(a) are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved, the Examiner is requested to contact the undersigned at the telephone number given below so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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